CLAIMS

What is claimed is:

1. A method for identifying highly synergistic combinations of biologically active agents comprising:

   i. selecting a library of potential actives in which synergy is sought ;

   ii. identifying a subset of components S(1) which display significant activity by testing each component of the library for activity in a Multi-Pathway High Throughput Assay that targets a biological end effect for which synergy is sought, and has a known useful range of detectability;

   iii. identifying a subset of binary mixtures designated S(2) that display synergy, by testing in the Multi-Pathway High Throughput Assay all or a portion of S(1) in binary mixtures with substantially each component of the library, including components that exhibited marginal or no activity in step ii), wherein the concentration of the S(1) component in the binary mixture is adjusted using a Single Component Scaling Protocol such that its activity is scaled back to a value $\lambda\ C_{Max,1}$, where λ is a scaling factor in the range from about 0.01 to 1, and $C_{Max,1}$ is the maximum activity reliably detectable at the top end of the useful range of the Multi-Pathway High Throughput Assay;

   iv. optionally identifying a subset of ternary synergistic mixtures, S(3) by testing in the Multi-Pathway High Throughput Assay all or a portion of the synergistic binary mixtures S(2) in combination with substantially each component of the library, including components that exhibited marginal or no activity in step ii), wherein the

concentrations of S(2) components used in the ternary mixture are adjusted using a Multiple Component Scaling Protocol such that the activity of S(2) is scaled back to a value $\lambda C_{Max,1}$;

v. optionally identifying a subset of N component synergistic combinations, designated S(N), wherein N is an integer greater than 3, by testing in the Multi-Pathway High Throughput Assay all or a portion of the synergistic mixtures containing (N-1) components, designated S(N-1) and identified in a manner analogous to step iv), in combination with substantially each component of the library including components that exhibited marginal or no activity in step ii), wherein the concentrations of the S(N-1) components used in this N component mixture are adjusted using the Multiple Component Scaling Protocol such that the activity of the S(N-1) mixture is scaled back to a value $\lambda C_{Max,1}$;

2. The method according to claim 1 wherein the subsets of S(1) through S(N-1) tested in combinations with members of the library contain only one or a small number of the components.

3. The method according to claim 1 or 2 wherein the library of potential actives comprises actives selected from the European EINECS list.

4. The method according to claim 1 wherein the library of potential actives comprises actives selected from the Cosmetics, Toiletries and Fragrance Association Dictionary of Compounds.

5. The method of claim 4 wherein the library of potential actives is a subset of the Cosmetics, Toiletries and Fragrance Association Dictionary of Compounds selected on the basis of being reported in that volume as having biological activity.

6. The method according to claim 1 wherein the library of potential actives comprises actives selected from at least two of the classes selected from the group consisting of retinol and its esters, retinol boosters, short peptides with or without hydrophobic modification or complexation with copper, minoxidil, natural products providing hypertensive activity, natural products containing antiandrogens, and chemicals capable of binding to the vitamin D receptor.

7. The method according to claims 1 wherein the Multi-Pathway High Throughput Assay targets the biological end effect of hair growth.

8. The method according to claim 7 wherein Multi-Pathway High Throughput Assay is the Isolated Hair Follicle Assay said assay comprising a multiplicity of cultures of individual hair follicles contained in a suitable growth medium wherein said follicles are removed from sectioned dermis in a viable state through the action of collagenase, and wherein hair growth is measured by a high throughput hair growth measurement technique which is not affected by the presence of contaminating tissues in the culture.

9. The method according to claim 8 wherein the high throughput hair growth measurement technique comprises the visualization of an incorporated fluorescent agent in the growing hair.

10. The method according to claim 7 wherein Multi-Pathway High Throughput Assay is the Low Temperature Whole Skin Organ Culture Assay, said assay comprising a multiplicity of miniaturized cultures of skin maintained in a serum free growth medium at a temperature below about 30, wherein said skin contains intact viable hair follicles below the sebaceous gland, and wherein hair growth is measured by a high-throughput measurement technique.

11. The method according to claim 10 wherein the high throughput hair growth measurement technique comprises the visualization of an incorporated fluorescent agent in the growing hair.

12. The method according to claim 1 wherein Multi-Pathway High Throughput Assay targets the biological effect of skin anti-aging.

13. The method according to claim 12 wherein the Multi-Pathway High Throughput Assay is the High Throughput Histology Organ Culture Assay said assay comprising the steps of simultaneously treating with test agents viable cultures of pieces of epidermis having at least a portion of their associated dermis intact, said pieces of epidermis contained in a multiple well assembly, wherein said treatment is followed by the embedding of large numbers of cultured skin pieces in a single block and histological sectioning of the entire assembly and the automated histology, histochemistry or immunohistochemisty of histological sections carried out in a high throughput manner.

14. The method according to claim 12 wherein the Multi-Pathway High Throughput Assay is the Topical Application High Throughput Histology Organ Culture Assay said assay comprising a culture of a sheet of viable epidermis and associated dermis, partitioned by the application of a surface barrier film into a pattern of isolated regions to which different test samples can be topically applied, followed after said treatment by the embedding of large numbers of cultured skin pieces in a single block and histological sectioning of the entire assembly and the automated histology, histochemistry or immunohistochemisty of histological sections carried out in a high throughput manner.

15. The method according to claim 1 wherein the Multi-Pathway High Throughput Assay is predictive of the resolution or prevention of acne.

16. The method of claim 15 where the Multi-Pathway High Throughput Assay is the Simulated Follicle P. Acnes Assay said assay comprising a culture of P. Acnes dispersed in a synthetic analogue of human sebum and held in an assembly comprising pieces of tubing of dimensions similar to the sebaceous duct, said assembly being exposed to antibacterial agents under realistic conditions and wherein growth of the surviving P. Acnes is measured by a high throughput measurement technique.

17. The method of claim 15 where the Multi-Pathway High Throughput Assay is the Inhibition of IL1-Induced Hypercornification Assay said assay comprising either a viable culture of miniature pieces of epidermis with at least a portion of associated dermis in a multiple well assembly, or a culture of a sheet of viable epidermis and associated dermis, partitioned by the application of a surface barrier film into a pattern of isolated regions to which different test samples can be topically applied, in either case using a culture medium containing a level of IL1 sufficient to cause hypercornification; followed by the embedding of large numbers of cultured skin pieces in a single block and histological sectioning of the entire assembly and the automated histology, histochemistry or immunohistochemisty of histological sections carried out in a high throughput manner.

18. The method according to claim 1 wherein the Multi-Pathway High Throughput Assay targets the biological end effect of dental plaque control.

19. The method according to claim 18 wherein Multi-Pathway High Throughput Assay is the Plaque Biofilm Regrowth Assay said assay comprising the growth of a plaque

biofilm in multiwell plates under conditions of medium replenishment approximately simulating chemostat conditions, followed by the partial removal of the biofilm using mechanical abrasion in the presence of the test agents and detection of the rate of regrowth of the biofilm, optionally using a disclosing agent, via automated image detection.

20. The method according to claim 1 wherein the Multi-Pathway High Throughput Assay targets the biological end effect of microbial growth inhibition or control.

21. The method according to claims 1 wherein the Multi-Pathway High Throughput Assay targets the biological end effect of skin pigmentation control.

22. The method according to claims 1 wherein the Multi-Pathway High Throughput Assay targets the biological end effect of sebum control.

23. The method according to claim 1 wherein the Multi-Pathway High Throughput Assay targets the biological end effect of dandruff control.

24. The method according to claim 1 wherein λ is less than or equal to 0.5.

25. The method according to claim 1 wherein λ is less than or equal to 0.2.

26. The method according to claim 1 wherein λ is less than or equal to 0.1.

27. The method according to claim 1 wherein N is an integer between 3 and 10.

28. A highly synergistic combination of biological agents providing a specific biological effect, said combination identified by the method of claim 1.

29. A highly synergistic combination according to claim 28 wherein the biological effect is hair growth stimulation.

30. A shampoo or hair conditioning composition containing the highly synergistic combination identified according to claim 29.

31. The composition of claim 30 wherein all components of the highly synergistic combination have an octanol/water partition coefficient higher than 100.

32. The composition of claim 30 wherein all components of the highly synergistic combination have an octanol/water partition coefficient higher than 1000.

33. A highly synergistic combination according to claim 28 wherein the biological effect is skin anti-aging.

34. A highly synergistic combination according to claim 28 wherein the biological effect is dental anti-plaque.

35. A highly synergistic combination according to claim 28 wherein the biological effect is the manipulation of skin pigmentation.

36. A highly synergistic combination according to claim 28 wherein the biological effect is sebum control.

37. A highly synergistic combination according to claim 28 wherein the biological effect is dandruff control.

38. A highly synergistic combination according to claim 28 wherein the biological end effect is the resolution or prevention of acne.

39. A highly synergistic combination according to claim 28 wherein the biological effect is microbial growth control.

40. A method for identifying agents that display maximum biological activity in hair growth comprising testing the agents in the Isolated Hair Follicle Assay, said assay comprising a multiplicity of cultures of individual hair follicles contained in a suitable growth medium wherein said follicles are removed from sectioned dermis in a viable state through the action of collagenase, and wherein hair growth is measured by a high throughput hair growth measurement technique and wherein said measurement technique is not affected by the presence of contaminating tissues in the culture.

41. The method according to claim 40 wherein the high throughput hair growth measurement technique comprises the visualization of an incorporated fluorescent agent in the growing hair.

42. A method for identifying agents that display maximum biological activity in hair growth comprising testing the agents in the Low Temperature Whole Skin Organ Culture Assay said assay comprising a multiplicity of miniaturized cultures of skin maintained in a serum free growth medium at a temperature below about 30 C wherein said skin contains intact viable hair follicles below the sebaceous gland,

and wherein hair growth is measured by a high-throughput hair growth measurement technique.

43. The method according to claim 42 wherein the high throughput hair growth measurement technique comprises the visualization of an incorporated fluorescent agent in the growing hair.

44. A method for identifying agents that display maximum biological activity in skin antiageing comprising testing the agents in the High Throughput Histology Organ Culture Assay said assay comprising the steps of simultaneously treating with test agents viable cultures of pieces of epidermis having at least a portion of their associated dermis intact,said pieces of epidermis contained in a multiple well assembly, wherein said treatment is followed by the embedding of large numbers of cultured skin pieces in a single block and histological sectioning of the entire assembly and the automated histology, histochemistry or immunohistochemisty of histological sections carried out in a high throughput manner.

45. A method for identifying agents that display maximum biological activity in skin antiageing comprising testing the agents in the Topical Application High Throughput Histology Organ Culture Assay said assay comprising a culture of a sheet of viable epidermis and associated dermis, partitioned by the application of a surface barrier film into a pattern of isolated regions to which different test samples can be topically applied, followed after said treatment by the embedding of large numbers of cultured skin pieces in a single block and histological sectioning of the entire assembly and the automated histology, histochemistry or immunohistochemisty of histological sections carried out in a high throughput manner.

46. A method for identifying agents that display maximum biological activity in controlling acne comprising testing the agents in the Simulated Follicle P. Acnes Assay said assay comprising a culture of P. Acnes dispersed in a synthetic analogue of human sebum and held in an assembly comprising pieces of tubing of dimensions similar to the sebaceous duct, said assembly being exposed to antibacterial agents under realistic conditions and wherein growth of the surviving P. Acnes is measured by a high throughput method.

47. A method for identifying agents that display maximum biological activity in controlling acne comprising testing the agents in the Inhibition of IL1 Induced Hypercornification Assay said assay comprising either a viable culture of miniature pieces of epidermis with at least a portion of associated dermis in a multiple well assembly, or a culture of a sheet of viable epidermis and associated dermis, partitioned by the application of a surface barrier film into a pattern of isolated regions to which different test samples can be topically applied, in either case using a culture medium containing a level of IL1 sufficient to cause hypercornification; followed by the embedding of large numbers of cultured skin pieces in a single block and histological sectioning of the entire assembly and the automated histology, histochemistry or immunohistochemisty of histological sections carried out in a high throughput manner.

48. A method for identifying agents that display maximum biological activity in controlling dental plaque comprising testing the agents in The Plaque Biofilm Regrowth Assay said assay comprising the growth of a plaque biofilm in multiwell plates under conditions of medium replenishment approximately simulating chemostat conditions, followed by the partial removal of the biofilm using mechanical abrasion in the presence of the test agents and detection of the rate of regrowth of the biofilm, optionally using a disclosing agent, via automated image detection.